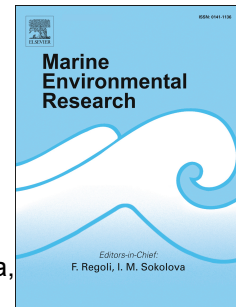


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Effects of the 2015 heat wave on benthic invertebrates in the Tabarca Marine Protected Area (southeast Spain)

Esther Rubio-Portillo^{a,b,*}, Andrés Izquierdo-Muñoz^b, Juan F. Gago^c, Ramon Rosselló-Mora^c, Josefa Antón^d, Alfonso A. Ramos-Esplá^{a,b}

^a Department of Marine Science and Applied Biology, University of Alicante, Alicante, Spain.

^b Centro de Investigación Marina de Santa Pola (CIMAR), University of Alicante-Santa Pola Town Council, Cabo de Santa Pola s/n, Alicante, Spain

^c Marine Microbiology Group, Department of Ecology and Marine Resources, Mediterranean Institute for Advanced Studies (IMEDEA, CSIC-UIB), Esporles, Spain

^d Department of Physiology, Genetics and Microbiology, University of Alicante, Alicante, Spain.

*Corresponding author: esther.portillo@ua.es

Abstract

In the late summer of 2015, extensive mortality of scleractinian corals, gorgonians, and sponges was observed in the Marine Protected Area of Tabarca (southeast Spain). Quantitative data indicated that at 25 meters depth the sea fan *Eunicella singularis* was the most affected species (50% of colonies affected by partial mortality); while in shallow waters more than 40% of the endemic scleractinian coral *Cladocora caespitosa* population showed tissue lesions that affected more than 10% of their surfaces. Other affected species were the scleractinian corals *Oculina patagonica* and *Phyllangia mouchezii*, the sea fan *Leptogorgia sarmentosa* and the sponge *Sarcotragus fasciculatus*. This mortality event coincided with an abnormal rise in seawater temperature in this region. Microbiological analysis showed a higher abundance of culturable *Vibrio* species in invertebrates exhibiting tissue lesions, which indicated that these opportunistic pathogens could be a key factor in the process.

Key words: Benthic invertebrates, Tabarca Marine Reserve, Mediterranean Sea; Mortality event

Introduction

The Mediterranean Sea is an enclosed miniature ocean, where the effects of global warming are likely to appear earlier and more intensely than in other more open oceans (Coll *et al.*, 2010) and several studies have confirmed such rapid warming of surface seawater. An increase of 0.04 °C \pm 0.01 °C year⁻¹ in sea surface temperature for the whole Mediterranean basin was detected in recent years (1982-2005; Diaz-Almela *et al.*, 2007). A recent study shows that during the last three decades the summer sea surface temperature has increased on average by 1.15°C, and the warming trends range from 0.25°C decade⁻¹ in the western basin to 0.65°C decade⁻¹ in the eastern basin (Marbá *et al.*, 2015).

Above normal sea temperatures have been linked to mass mortality outbreaks in coastal ecosystems during the past few decades (Harvell *et al.*, 1999). In the Mediterranean Sea, even short duration temperature anomalies have had serious consequences on marine diversity. In the western Mediterranean, positive thermal anomalies during summer 1999 and 2003 resulted in the most catastrophic mass mortality events observed until now. The 1999 episode was the first major regional event recorded and at least 30 benthic species along hundreds of kilometers of the northwest Mediterranean coastline were affected (Cerrano *et al.*, 2000; Perez *et al.*, 2000; Bensoussan *et al.*, 2010; Crisci *et al.*, 2011). The 2003 heat wave struck a much larger area, almost the whole western Mediterranean, Adriatic and central Mediterranean, and 25 benthic species belonging to the Phyla Cnidaria, Porifera, Bryozoa, and Mollusca were affected (Garrahou *et al.*, 2009).

The direct causes of these events in the Mediterranean remain unknown, although climatic conditions were similar during their occurrence, including temperatures 3–4 °C above average and prolonged water column stability and stagnation in late summer. Exposure to high

temperatures causes physiological stress to marine invertebrates under summer low-food conditions and this appears to be the main driver of severe mass mortality events (Coma *et al.*, 2009). These circumstances could make benthic organisms more susceptible to opportunistic and pathogenic microorganisms. Most marine bacterial pathogens (such as *Vibrio* spp.) are temperature sensitive, with an expected increase in abundance and virulence under long-term high sea-surface temperatures (Vezzulli *et al.*, 2010; Kimes *et al.*, 2012). Therefore, rising global temperatures due to climate change drive a geographical expansion of pathogens and the spread of disease outbreaks (Harvell *et al.*, 1999, 2002, Baker-Austin *et al.*, 2013; Vezzulli *et al.*, 2013).

In the Mediterranean Sea, two *Vibrio* pathogens linked to Cnidaria diseases have been identified up to now: *Vibrio coralliilyticus*, involved in mass mortality events of the purple sea-fan *Paramuricea clavata* (Bally and Garrabou, 2007; Vezzulli *et al.*, 2010), and *V. shiloi*, a later heterotypic synonym of *V. mediterranei* (Thompson *et al.*, 2001), identified as the causative agent of mass bleaching events in *Oculina patagonica* (Kushmaro *et al.*, 1996, 1997). Other recent studies also point to the implication of these two pathogens in *O. patagonica* bleaching (Mills *et al.*, 2013; Rubio-Portillo *et al.*, 2014a). Although studies on mass mortalities of other benthic invertebrates in the Mediterranean Sea and the involvement of *Vibrio* pathogens are very scarce, Martin *et al.*, (2002) and Stabili *et al.*, (2012) described a proliferation of *Vibrio* spp. in diseased specimens of *Eunicella cavolonii* and *Ircinia variabilis*, respectively.

In summer 2015, the Spanish Mediterranean suffered an exceptional heat wave with air temperature records 1.5°C above the seasonal averages from 1981-2010. This was the second hottest summer since 1961, only exceeded by summer 2003. This anomalous event began at the end of June 2015 and lasted through all July. The latter month was the hottest July recorded, with a monthly average of 26.5°C. In fact, the southeast Iberian Peninsula, where the Tabarca Marine Protected Area (MPA) is located, reached a new all-time record for the July average

temperature (28.2°C). This was 2.7°C higher than the monthly average for the early 1900s (State Meteorological Agency, www.aemet.es).

Hence, the main goal of this paper is to assess the effects of extraordinary episodes of high temperatures on marine benthic invertebrates, using as example the 2015 heat wave on the MPA of Tabarca. We provide data on the main affected species, the intensity of the impact and the depth range affected, as well as the presence of *Vibrio* pathogens in marine invertebrates with signs of tissue loss. Here, even though we cannot provide quantitative data for all species, we confirm that the mortality signs observed during and after that summer are unusual and may well be related to the impact of the heat suffered in July in this region.

Material and Methods

Study Area

This study was carried out during the summer and autumn of 2015 in the Marine Protected Area of Tabarca (south-western Mediterranean Sea, Spain; Fig. 1). This MPA, created in 1986, was the first in Spain (Ramos, 1985) and has been monitored since 2011 within the framework of the Tropical Signals Program (Moschella, 2008), whose aim is to evaluate the effects of tropicalization of the Mediterranean Sea, as a consequence of climate warming, using reliable representative biological macrodescriptors. This study has been conducted in the MPA because this kind of environment is better suited for observing climate change effects than more disturbed regions, where there is normally more interference from other factors such as anthropogenic stressors (Otero *et al.*, 2013).

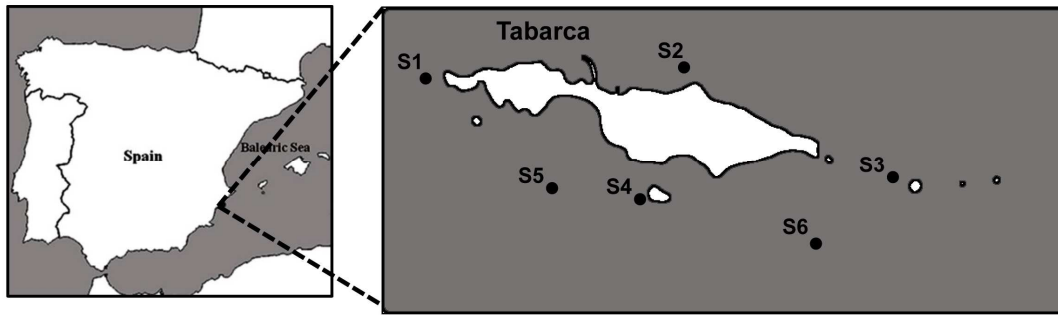


Fig. 1. Surveyed locations in the Tabarca Marine Protected Area (western Mediterranean) within the Tropical Signals program.

Temperature datasets

Seawater temperature daily data series (2006-2015) taken at 3m depth from Alicante (2007-2014) and Cabo de Palos buoys, at 60km from Tabarca Island, in the Spanish port network (<http://www.puertos.es>) were analyzed. Records obtained from the two buoys showed that trends in the thermal anomalies are very similar in both areas, although always somewhat more pronounced off Alicante (Fig. 2). The Cabo de Palos data, showing the highest resolution covering the longest time period of seawater temperature datasets in this region, were used to calculate temperature anomalies throughout summer 2015 in this region (relative to the 2006–2014 baseline).

In situ temperature data were collected daily at 25 m (July–November 2015) depth by a temperature data logger (HOBO ProV2) located at one of the MPA buoys (N 38° 10′ 94″ N, 00° 29′ 60″ W). Seawater temperature was also measured each ten minutes at 16 m depth in Alicante bay, at 17 km from Tabarca island, by a salinity and temperature sensor (Alec Electronics COMPACT-CT).

Assessment of impact of the 2015 heat wave on benthic marine organisms

Qualitative and quantitative data on invertebrate health status were acquired to assess the impact of the summer 2015 heat wave. A list of 9 possible affected species was obtained, including mainly sponges, corals and gorgonians that showed tissue necrosis or recent epibiosis (Table 1). The quantitative analysis was focused on the gorgonians *Eunicella singularis* and *Leptogorgia sarmentosa*, the scleractinian corals *Cladocora caespitosa* and *Oculina patagonica*, and the sponge *Sarcotragus fasciculatus* (= *Ircina fasciculata*), since these were the most abundant species affected in the study area. Other species, such as scleractinian coral *Phyllangia mouchezii* and *Balophyllia europaea*, the sponge *Sarcotragus spinolosus* and the bryozoan *Myriapoda truncate*, were also observed during the sampling campaigns. Four sites (S1-S4 in Fig. 1) were studied between 3 and 10m depth to assess the impact on shallow scleractinian corals and sponges, and 2 more sites (S5 and S6 in Fig. 1) were used for gorgonians at 25 meters depth. At each site, surveys were conducted along four 25m long transects and the total number of specimens of each species was recorded. Details were taken of specimens showing necrosis or epibiosis of more than 10% of their surface tissue, and also if the colonies were partially or completely affected (RAC/SPA-UNEP/MAP 2014). Furthermore, results obtained at site 5 during summer 2015 were compared with previous years, 2012 and 2014.

Enumeration of *Vibrios* and data treatment

Three water samples from the surface and 25 meters depth and four small pieces (~2 cm²) from healthy specimens and those with tissue lesions of *E. singularis*, *L. sarmentosa*, *C. caespitosa*, *O. patagonica* and *S. fasciculatus* were removed from Tabarca MPA in September 2015. Samples were transported to the lab and gently washed three times with 50 ml of sterile filtered seawater (SFSW) to remove non-associated bacteria. Approximately 2 g (wet weight) of tissue of each sample was crushed with 5ml SFSW using a mortar and pestle. For scleractinian corals, after allowing the skeletal matrix to settle, the supernatant (largely containing crushed tissue)

was removed and kept for *Vibrio* counts. For plate counts of *Vibrio* spp., 100 µl of 10-fold serial dilutions of seawater samples and crushed tissue were prepared in SFSW, plated on the *Vibrio*-selective medium thiosulphate citrate bile sucrose (TCBS) agar (Pronadisa, Spain), and incubated at 30°C for 48 h. For each host species, differences in quantification of *Vibrio* abundance were tested using one-factor (health status) ANOVA (analysis of variance). Before calculating ANOVA, heterogeneity of variance was tested using Cochran's C-test.

MALDI-TOF MS analyses of *Vibrio* strains

A recent study has demonstrated that MALDI-TOF MS profiling can be used for rapid identification of *Vibrio* environmental isolates (Erler et al., 2014). Consequently we carried out an initial screening for *Vibrio* strains with MALDI-TOF. A total of 100, isolated from the 5 invertebrate species (see above), were selected on the basis of their color, size and morphology in order to identify the presence of possible *Vibrio* pathogens in marine invertebrates affected by the 2015 heat wave, using whole cell biomass as previously published (Viver et al., 2015). For pathogen identification, three reference strains (*V. mediterranei* = *V. shiloi* AK-1 = CECT 7873, *V. mediterranei* CECT 623, and *V. coralliilyticus* LMG 20984^T). The matching scores of these strains with the reference strains were divided into ranges: highly probable species identifications (>2.3), probable species identifications (2.0–2.3), reliable genus identifications (1.7–2.0) and unreliable identifications (<1.7), and the profiles of *Vibrio* isolates with matching scores up to 2 with these reference strains were grouped into clusters

Results and discussion

Thermal anomalies

The mass mortality events observed in the Mediterranean Sea were likely triggered by two types of thermal anomaly (Crisci *et al.*, 2011). The first type is characterized by short episodes (up to 5 days in duration) with mean seawater temperatures around 27 °C and high intra-day

variability, while the second presents long periods (up to 40 days in duration) with a sea temperature of up to 24 °C and low intra-period variability. In the region of southeast Spain, coastal waters underwent a significant warming event in the summer of 2015, staying at 24°C for over 80 days and the maximum temperature observed during this heat wave reached 28.23°C. Compared with the preceding 9 years, the warming anomaly during July and August was of 2°C or more and persisted for approximately 6 weeks (Fig. 2).

At 16 meters depth, seawater temperature was at 24°C for 29 consecutive days and at 25m temperature was above 24°C at the beginning of September (Fig. 3). Usually, 25m corresponds to the seasonal intermediate thermocline level but the fact that seawater temperature at this depth was similar to the shallow water temperature indicates that the thermocline remained below 25m depth during the whole summer. Therefore, the region of southeast Spain showed a long-term anomaly in 2015 with warm mean seawater temperatures of approximately 24°C associated with low intra-period variability.

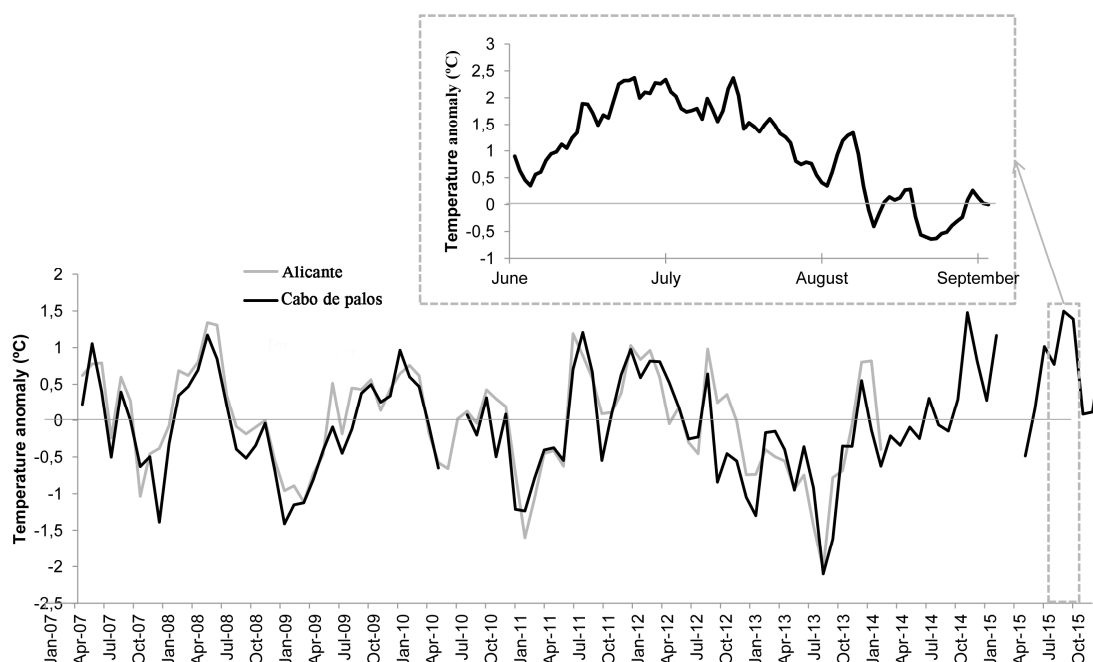


Fig. 2. Monthly temperature anomalies during 2007-2015 for Alicante and Cabo de Palos (°C with respect to the mean calculated for the entire period) and summer temperature anomalies for 2015 (°C relative to means of the preceding 9 years) generated from Cabo de Palos buoy series.

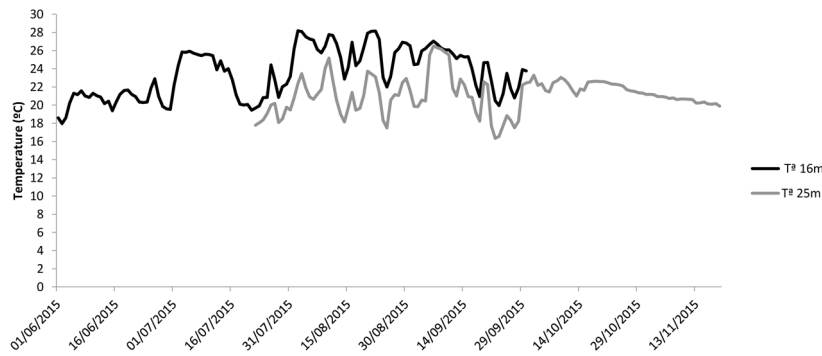


Fig. 3. Seawater temperature at 16m depth in Alicante bay and at 25m in the Tabarca Marine Protected Area.

Affected species

After the summer of 2015 a mortality event occurred, affecting coralline algae at depths ranging from 0 to 30m depth in the northwestern Mediterranean Sea, which has been related to positive seawater thermal anomalies (Hereu and Kersting, 2016). Within Tabarca MPA, some important hard-bottom bioengineering species belonging mainly to Class Anthozoa (Table 1) showed signs of mortality in the depth range between 3 and 25m. Although our shallow temperature data are too incomplete to draw any firm conclusions, together with data recorded at 25m depth they indicate the mortality event observed in Tabarca was registered concomitantly with the occurrence of temperatures higher than average.

Table 1. Species affected during the 2015 mass mortality in the Tabarca Marine Protected Area . (A): Species affected by tissue lesions; (NA) species not affected, with only two or three colonies affected; (NO) not enough observations

Species	Depth in meters	Nº transects	Nº colonies	Species status (% of colonies affected mean \pm SE)
Alcyonacea				
<i>Eunicella singularis</i>	20-25	8	73	A (53.82 \pm 4.36)
<i>Leptogorgia sarmentosa</i>	20-25	8	38	A (41.11 \pm 8.40)
Scleractinia				
<i>Cladocora caespiosa</i>	3-10	12	25	A (47.22 \pm 11.01)
<i>Oculina patagonica</i>	3-10	12	44	A (30.09 \pm 6.27)
<i>Phyllangia mouchezii</i>	20-25	8	3	A, NO
<i>Balanophyllia europaea</i>	3-10	12	21	NA

Demospongiae				
<i>Sarcotragus fasciculatus</i>	3-10	16	212	A (42.60 ± 4.22)
<i>Sarcotragus spinolosus</i>	3-10	16	39	NA
Bryozoa				
<i>Myriapoda truncata</i>	3-10	12	3	NO

At 3 meters depth, *S. fasciculatus* was affected by the rise in seawater temperature in summer 2015, the first record of disease signs was noted at the beginning of July and by the end of September more than 40% of the population (Table 1) was partially affected by tissue lesions, evident from the disappearing ectosome and the spongine skeleton becoming visible (Fig. 4a). Furthermore, skeletons of completely dead specimens were occasionally observed on the beaches of the MPA (personal observations), likely as a result of this event. Extensive mortalities in *S. fasciculatus* populations have been previously related to thermal anomalies, with similar characteristics to those reported here, for locations off southern Spain (Maldonado *et al.*, 2010), the Balearic Islands and Corsica (Cebrian *et al.*, 2011), the southern Adriatic and Ionian Sea (Stabili *et al.*, 2012), and the northern Adriatic Sea (Di Camilo *et al.*, 2013).

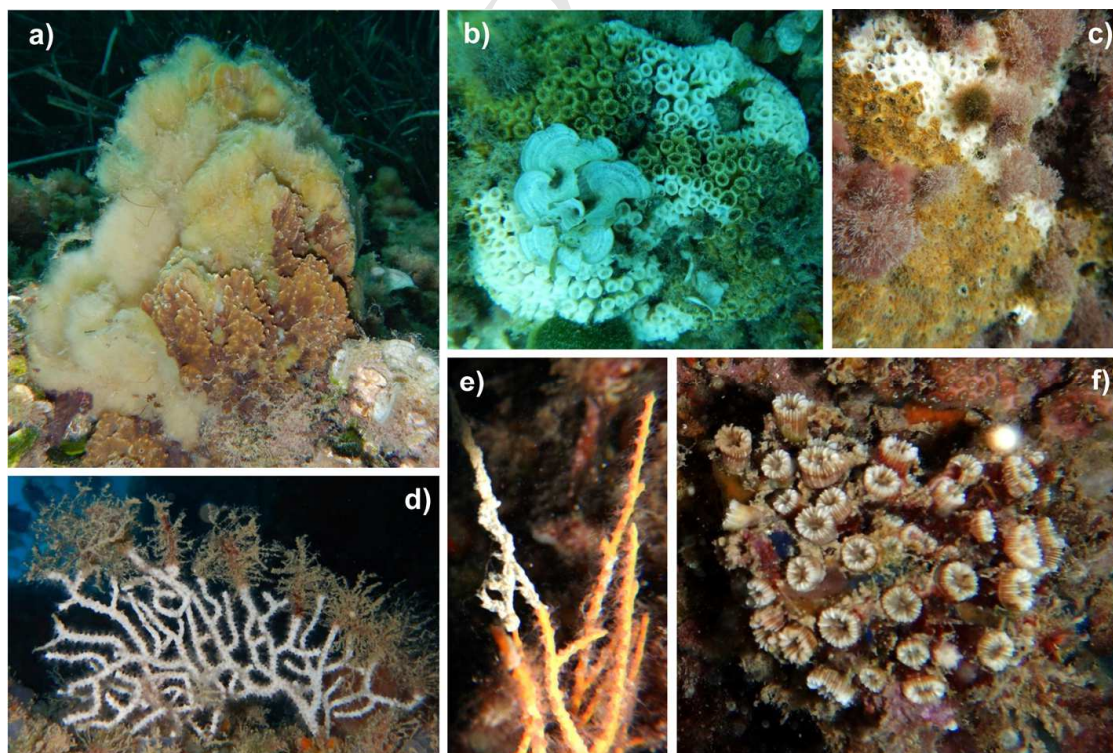


Fig. 4. Tissue lesions in affected invertebrate species during the 2015 mass mortality event in the Tabarca Marine Protected Area. a) *Sarcotragus fasciculatus*; b) *Cladocora caespitosa*; c) *Oculina patagonica*; d) *Eunicella singularis*; e) *Leptogorgia sarmentosa*; f) *Phyllangia mouchezii*.

Among the shallow scleractinian corals present in the MPA, the endemic *C. caespitosa* (Fig. 4b) was the shallow species most affected by the mortality event, resulting in the total or partial death of almost 50% of its population (Table 1). This species also showed tissue necrosis signs in other areas along the east coast of Spain, such as Columbretes MPA (Kersting, pers. comm.), where this species has suffered recurrent tissue necrosis since 2003, following summers in which positive thermal anomalies have been recorded (Kersting *et al.*, 2013). The scleractinian coral *O. patagonica* showed lower percentages of tissue damage than *C. caespitosa* (Table 1). These were similar to those detected in the same study area in 2011 (Rubio-Portillo *et al.*, 2014b), when temperatures over 26°C were maintained for 54 consecutive days (Rubio-Portillo *et al.*, 2014b). However, in the 2015 mortality event, *O. patagonica* showed tissue necrosis (Fig. 4c) instead of the bleaching observed in 2011.

Nevertheless, the solitary scleractinian coral *Balanophyllia europaea* is a common species in shallow waters in Tabarca MPA and was not affected by this event, since all its colonies showed normal coloration (n=21). However, this species has undergone mass bleaching followed by tissue necrosis in previous years during thermal anomalies, such as those observed off the coast of Genoa and Spain in 2003 (Garrahou *et al.*, 2009; Kersting and Templado, 2006), and in the Adriatic Sea in 2003, 2009 and 2012 (Kružić and Popijač, 2014). These differences are probably because populations exposed to contrasting temperature regimes can exhibit different degrees of resilience to global warming (Hughes *et al.*, 2003), as demonstrated in other Mediterranean species (Linares *et al.*, 2005).

At 25 meters depth, the most affected species were the gorgonians *E. singularis* and *L. sarmentosa* (Table 1). These two species displayed a 4 to 8-fold increase in affected colonies compared to previous years (Table 2), which is an excellent descriptor to quantify the impact of the mass mortality event in Tabarca MPA. Both species firstly showed tissue necrosis that resulted in denuded axes, and finally at the end of September and early October their axes began to show epibiosis (Fig. 4d and 4e). Such a pattern could result from exposure to high temperatures that reduce the fitness of Mediterranean gorgonians as a consequence of a reduction in their metabolic activity, as previously observed in experimental aquaria by Previati *et al.* (2010).

Table 2. Mean percentage of colonies with more than 10% of colony surface showing tissue lesions from the same gorgonian populations (study site S5, see Fig. 1) in different years. (NA) not affected

Species	Survey years	N° colonies	% Affected colonies
<i>Eunicella singularis</i>	2012	25	6.60 ± 3.88
	2014	35	7.88 ± 5.14
	2015	31	49.87 ± 3.65
<i>Leptogorgia sarmentosa</i>	2012	10	NA
	2014	13	8.9 ± 3.52
	2015	12	41.66 ± 4.81

Two specimens of the scleractinian coral *Phyllangia mouchezii* also showed tissue loss during the heat wave (Fig. 6f), but this species was rare at the sampling sites (n=3) and data was insufficient to determine the degree of injury in this species as a consequence of heat stress. Tissue necrosis of *P. mouchezii* was not observed in the severest mass mortality events that affected the western Mediterranean basin in 1999 and 2003, nor in 2012 in the Levantine area (Jimenez *et al.*, 2014), which suggests that this species is probably more thermotolerant than other Mediterranean coral species.

Detection of Vibrio spp. in the affected invertebrates

Besides anomalous temperature conditions, mortality due to thermo-dependent pathogens cannot be ruled out, considering that experimental and field data confirm that the rise of seawater temperature promotes pathogen virulence and/or favors host susceptibility (Martinez-Urtaza et al., 2010; Vezzulli et al., 2010; Kimes et al., 2012; Gallana et al., 2013; Maynard et al., 2015). In marine habitats, the spread of *Vibrio*-related diseases has been identified as an emerging global concern correlated to rising seawater temperatures (Cervino et al., 2004; Martinez-Urtaza et al., 2010; Vezzulli et al., 2012, 2013; Baker-Austin et al., 2013). Furthermore, *Vibrio* species were recently identified as specific etiological agents in mass mortalities of benthic invertebrates in the Mediterranean. *V. coralliilyticus* has been implicated in diseases affecting the gorgonian *Paramuricea clavata* (Bally and Garrabou 2007) and *V. mediterranei* in *O. patagonica* bleaching (Kushmaro et al., 1996; 1997; Rubio-Portillo et al., 2014a). Vibrios have also been related to the appearance of disease signs in the genus *Ircinia* (= *Sarcotragus*) in the Mediterranean Sea (Stabili et al., 2012), although in this case Koch's postulates were not satisfied to prove etiology.

In order to assess the possible relationship between rising seawater temperature, presence of *Vibrio* spp. and the appearance of tissue lesions in marine invertebrates, we collected water and tissue samples from healthy and diseased invertebrates and used them to inoculate a *Vibrio*-specific culture medium. In seawater samples, *Vibrio* spp. reached $3.39 \times 10^4 \pm 4.01 \times 10^3$ CFU l⁻¹ and $4.45 \times 10^3 \pm 1.95 \times 10^2$ CFU l⁻¹ at 3 and 25 meters depth, respectively. The cultivable *Vibrio* counts at 3 meters were about 10-fold higher than values detected in the same area in 2011, when a bleaching event in *O. patagonica* was recorded (Rubio-Portillo et al., 2014a). The higher occurrence of *Vibrio* spp. in shallow waters is probably correlated with temperature, as observed in previous studies that showed *Vibrio* spp. abundance in seawater was higher during warmer months (Vezzulli et al., 2010). *Vibrio* counts in invertebrate samples collected during the studied mortality event were higher in specimens that showed tissue lesions in all species, but abundances were significantly higher in *E. singularis*, *S. fasciculatus*, *O. patagonica* and *C. caespitosa* (ANOVA, $p < 0.005$), with a 5 to 15-fold increase in affected colonies compared to

specimens that did not show signs of disease (Fig. 5). Therefore, the increase in *Vibrio* numbers appears to be correlated with the health status of the benthic invertebrates studied in this work. It is therefore likely that these bacteria were involved as etiological agent in the mortality event observed in the Tabarca MPA during summer 2015. In fact, among the 100 selected *Vibrio* isolates analyzed by MALDI-TOF, 11 isolates from diseased specimens of *E. singularis*, *S. fasciculatus* and *O. patagonica* showed high similarities with *V. mediterranei*, with scores ranging from 2 to 2.4 (Fig. 6 and Table 3). The identification of the majority of the isolates as *V. mediterranei* is in accordance with the type strain AK-1 of the species being previously identified as the causative agent of mass bleaching events in *O. patagonica* (Kushmaro *et al.*, 1997). Another 4 strains were also thus related to the coral pathogen *V. coralliilyticus*, although all were isolated from apparently healthy organisms (Table 3).

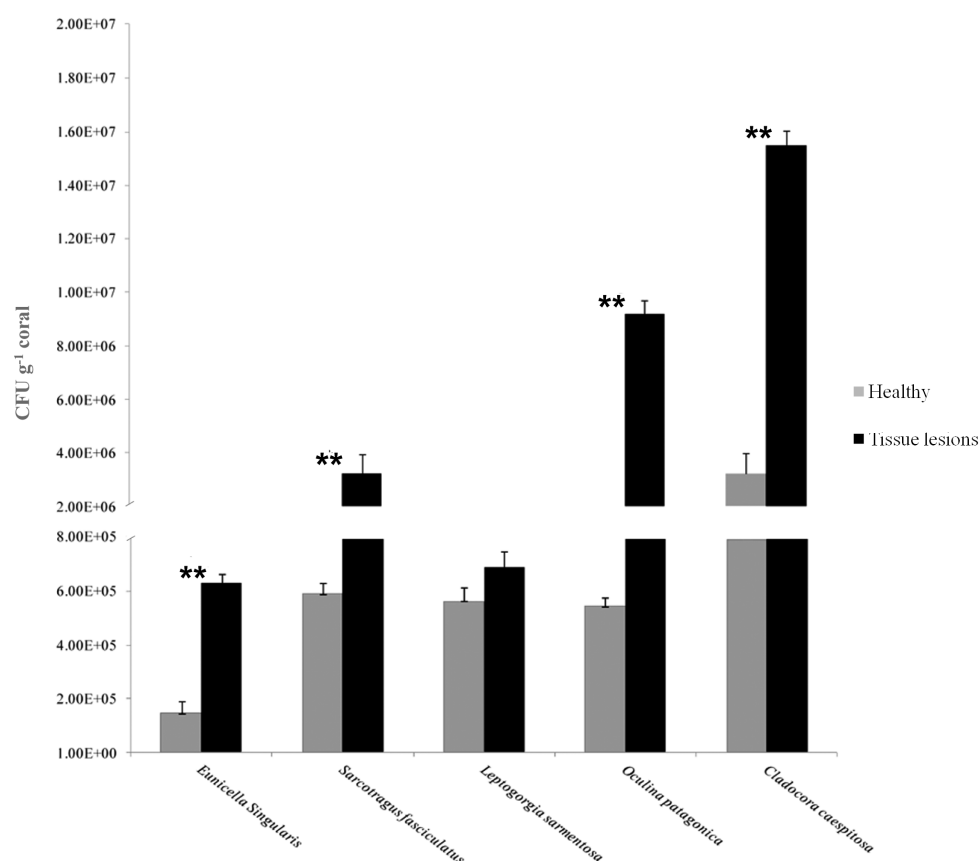


Fig. 5. Concentration of culturable vibrios in healthy and diseased colonies of marine invertebrates. ** $p < 0.05$

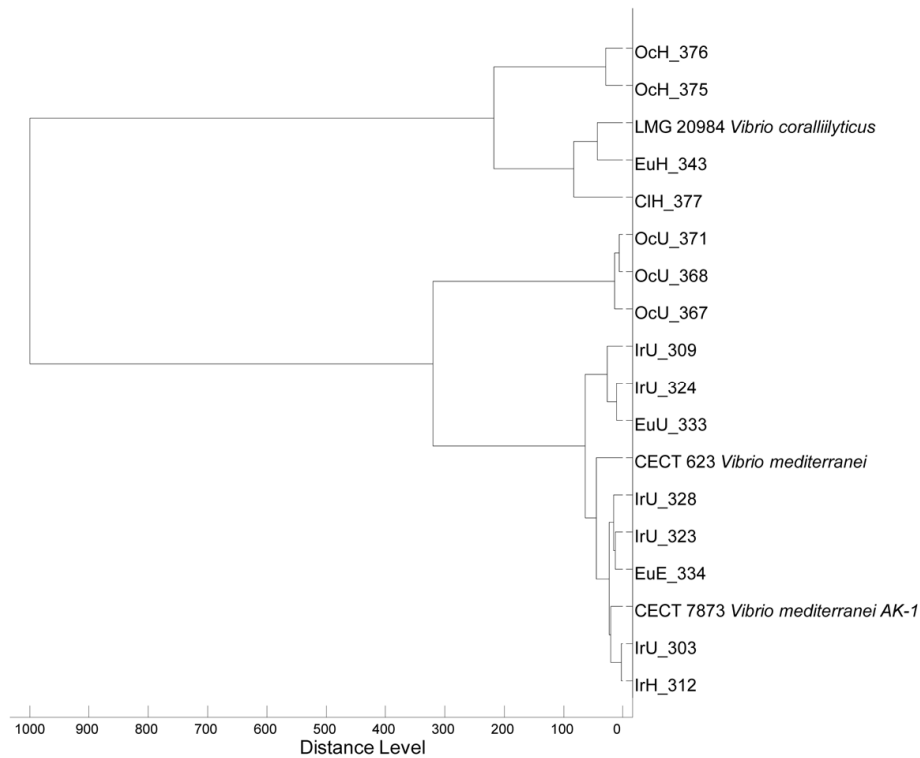


Figure 6. Dendrogram of relatedness between *Vibrio* isolates from marine invertebrates and type strains LMG20984 *V. coralliilyticus*, CECT 623 *V. mediterranei*, CECT 7873 *V. mediterranei* AK-1 as reference.

Table 3. Strains exhibiting high MALDI-TOF mass relatedness together with type strains LMG20984 *V. coralliilyticus*, CECT 623 *V. mediterranei*, CECT 7873 *V. mediterranei* AK-1.

Strain ID	Stain origin		Reference strain	Score
	Invertebrate specie	Health status		
IrU_323	<i>S. fasciculatus</i>	Tissue lesions	CECT 7873 <i>V. mediterranei</i>	2.51
IrU_328	<i>S. fasciculatus</i>	Tissue lesions	CECT 7873 <i>V. mediterranei</i>	2.471
EuE_334	<i>E. singularis</i>	Healthy	CECT 7873 <i>V. mediterranei</i>	2.461
IrH_312	<i>S. fasciculatus</i>	Healthy	CECT 7873 <i>V. mediterranei</i>	2.437
IrU_303	<i>S. fasciculatus</i>	Tissue lesions	CECT 7873 <i>V. mediterranei</i>	2.349
IrU_324	<i>S. fasciculatus</i>	Tissue lesions	CECT 623 <i>V. mediterranei</i>	2.299
EeU_333	<i>E. singularis</i>	Tissue lesions	CECT 7873 <i>Vibrio mediterranei</i>	2.247
EuH_343	<i>E. singularis</i>	Healthy	LMG 20984 ^T <i>V. coralliilyticus</i>	2.187
CIH_377	<i>C. caespitosa</i>	Healthy	LMG 20984 ^T <i>V. coralliilyticus</i>	2.163
IrU_309	<i>S. fasciculatus</i>	Tissue lesions	CECT 7873 <i>V. mediterranei</i>	2.134
OcH_375	<i>O. patagonica</i>	Healthy	LMG 20984 ^T <i>V. coralliilyticus</i>	2.08
OcH_376	<i>O. patagonica</i>	Healthy	LMG 20984 ^T <i>V. coralliilyticus</i>	2.072
OcU_367	<i>O. patagonica</i>	Tissue lesions	CECT 7873 <i>V. mediterranei</i>	2.059
OcU_368	<i>O. patagonica</i>	Tissue lesions	CECT 7873 <i>V. mediterranei</i> AK-1	2.032
OcU_371	<i>O. patagonica</i>	Tissue lesions	CECT 7873 <i>V. mediterranei</i>	2.006

Conclusion

The mass mortality event in the summer of 2015 is the first recorded in the Marine Protected Area of Tabarca. As in other mortality events in the Mediterranean Sea during recent decades, cnidarians and sponges were the most affected groups. However, it cannot be ascertained whether the rise in seawater temperature had only a direct effect on invertebrates or has a potential multifactorial origin, as suggested previously (Garrahou *et al.*, 2009; Kersting *et al.*, 2013, 2015; Rodolfo-Metalpa *et al.*, 2014). Probably these events are combined with the presence of potential microbial pathogens, which considerably increase their abundance with temperature. This work shows a relationship between seawater temperature, presence of *Vibrio* coral pathogens and the appearance of benthic invertebrate tissue lesions. Considering the present warming trend in the Mediterranean, new mass mortality events may occur in the near future. Therefore, programs monitoring vulnerable populations of gorgonians, corals and sponges would be best combined with physical and microbial parameters. These programs should be established in Marine Protected Areas, which are potentially resilient sites (or refugia) for these threatened species.

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Highlights

In the summer of 2015 a mass mortality event was recorded in the Marine Protected Area of Tabarca.

This mortality event coincided with an abnormal rise in seawater temperature in the region.

Opportunistic *Vibrio* pathogens were detected in invertebrates tissue lesions.